(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Peroxidase (POD) Activity Assay Kit

Catalog No.: BC00018

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

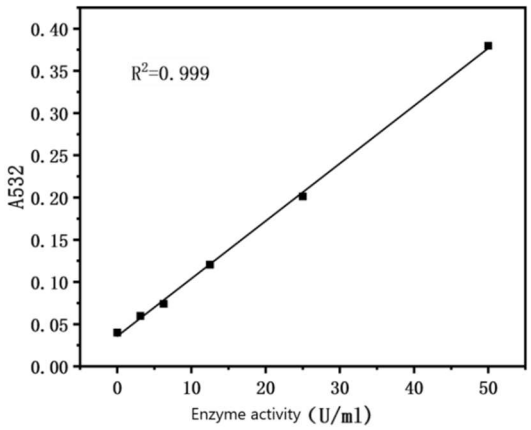
Product Name	Peroxidase (POD) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, cell and other samples
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (240 nm, 532 nm)

Product Introduction

This kit detects the activity of peroxidase (POD) in cells, tissues or other samples through colorimetric reaction. Peroxidase is a common enzyme that plays an important role in redox in organisms and is widely distributed in the liver, kidneys and red blood cells.

Principle

Under conditions where hydrogen peroxide is relatively abundant, peroxidase can catalyze the decomposition of hydrogen peroxide into water and oxygen. Hydrogen peroxide can oxidize a chromogenic substrate under the catalysis of peroxidase, resulting in a red product with a maximum absorption wavelength of 532nm. By measuring the absorbance at A532 or a similar wavelength and comparing it with a standard curve made from peroxidase standards, the peroxidase activity in the sample can be calculated.
The figure below shows the A532 readings for peroxidase standards detected by this kit.



Components

Components	Size (100T)	Storage
Peroxidase Assay Buffer	60ml	-20℃, can also be stored at 4℃ after opening.
Hydrogen Peroxide (approximately 1M)	5ml	-20℃
Chromogenic Substrate	20ml	-20℃
Peroxidase (50U/ml)	200μl	-20℃
Microplate	1 plate	RT
Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20°C for 12 months.

Preparation

- **Sample handling**

Lyse cells or tissues with an appropriate lysis solution. Dilute the lysed samples with the peroxidase assay buffer provided in this kit, and add at least an equal volume of peroxidase assay buffer to the lysed samples. The dilution factor should be adjusted according to the protein concentration of the samples, and common dilutions such as 1, 10, 20, 50 times can be used for initial attempts to ensure that the sample detection absorbance is within the linear range.

- **Preparation of the kit**

1. Prepare 250mM hydrogen peroxide solution

The hydrogen peroxide provided with this kit has an approximate concentration of 1M. Due to the poor stability of hydrogen peroxide, its actual concentration should be determined before use. Dilute the approximately 1M hydrogen peroxide with the peroxidase assay buffer provided in this kit by 100 times to achieve a hydrogen peroxide concentration of about 10mM. Measure A240. The determination of A240 can be done using any of the following methods:

a. Ordinary UV spectrophotometer method: Use UV spectrophotometer with cuvette holder, NanoDrop 2000C, NanoDrop One^{c}, QuickDrop and other instruments, with quartz cuvettes. Determine the path length of the cuvette, which is generally 1 cm. The concentration of hydrogen peroxide detected by the cuvette is closest to the actual concentration.
b. Micro-volume UV spectrophotometer method: such as NanoDrop 2000, NanoDrop One, QuickDrop, Varioskan with ultra-micro detection plate μDrop Plate and other instruments. Determine the path length: For NanoDrop 2000, NanoDrop One, etc., it is necessary to cancel the "automated path length", and the path length is generally 0.1 cm; the path length of Varioskan's ultra-micro detection plate μDrop Plate is generally 0.05 cm. For the specific path length of the micro-volume UV spectrophotometer, please refer to the instrument parameters.
c. 96-well UV microplate reader method (capable of detecting 240nm wavelength): Determine the optical path length based on the parameters of the 96-well plate, which is generally 0.552 cm for a 200-microliter sample (sample volume divided by the cross-sectional area of a single well in a 96-well plate). It is generally recommended to use a dedicated 96-well UV detection plate (such as a 96-well UV plate). If a UV detection plate is not available, a regular 96-well plate can be used, but since it is not specifically designed for UV detection, it will have a very high UV absorption signal. Therefore, it is necessary to set up wells with an equal amount of double-distilled water as a blank control (generally, 200 μl of water in such a 96-well plate has an A240 of around 3.8), and this blank control must be subtracted during calculation. When using a non-UV detection plate, due to the limited detection range of the 96-well microplate reader at 240 nm, it is recommended to dilute the hydrogen peroxide to about 10 mM before measuring the concentration.

Note: All of the above methods require setting up an equal amount of double-distilled water as a blank control, and this blank control must be subtracted during the calculation.

The formula for calculating the concentration of hydrogen peroxide is: $c=A/(\varepsilon \times b)$. Where: c is the sample concentration (in units of mol/L or M); A is the absorbance value; ε is the wavelength-dependent molar extinction coefficient (in units of $L \times mol^{-1} \times cm^{-1}$ or $M^{-1} \times cm^{-1}$), and the molar extinction coefficient of hydrogen peroxide is $43.6 M^{-1} cm^{-1}$; b is the path length (in units of cm).

Therefore: Hydrogen peroxide concentration (M) = A240/(43.6×b); that is: Hydrogen

peroxide concentration (mM) = 22.94×A240/b, which allows us to calculate the actual concentration of hydrogen peroxide provided by this kit. Then, based on the actual concentration of hydrogen peroxide, dilute and prepare a 250mM hydrogen peroxide solution with the peroxidase assay buffer.

2. Prepare 3.75mM hydrogen peroxide solution

Dilute and prepare 3.75mM hydrogen peroxide solution using the peroxidase assay buffer based on the actual hydrogen peroxide concentration obtained from measurement.

3. Dissolve the chromogenic solution

Dissolve the chromogenic substrate in an ice bath, aliquot as needed before use, and avoid repeated freezing and thawing. Keep other reagents on ice for standby.

Operation process

1. Determination of the Standard Curve

(1) Dilute peroxidase to 50, 25, 12.5, 6.25, 3.125, and 0 (blank well) U/ml for later use.

(2) Prepare standard well chromogenic working solution: mix the diluted peroxidase standards with the chromogenic substrate at a ratio of 1:1000. For example, mix 1μL of peroxidase with 1000μL of chromogenic substrate.

(3) Add 4μL of the above diluted 3.75mM hydrogen peroxide into the plate wells, then add 200μL of chromogenic working solution.

(4) Incubate at room temperature for 15 minutes and measure the A532 absorbance.

2. Determination of samples

(1) Prepare measurement well chromogenic working solution: mix the sample to be tested with the chromogenic substrate at a ratio of 1:1000. For example, mix 1μL of the sample with 1000μL of chromogenic substrate.

(2) Add 4μL of 3.75mM hydrogen peroxide into the plate wells, then add 200μL of the mixed solution of the sample and chromogenic solution.

(3) Incubate at room temperature for 15 minutes and measure the A532 absorbance, or measure the absorbance at a nearby wavelength.

Operation Table is as follows:

	Standard well	Measurement well
3.75mM hydrogen peroxide (μL)	4	4

Different concentrations of standard well chromogenic working solution (μL)	200	--
Measurement well chromogenic working solution (μL)	--	200
Incubate at room temperature for 15 minutes, measure the OD values at 532nm in each well.		

Calculation

1. Use the standards to plot the standard curve and calculate the relationship between peroxidase activity (x) and A532 (y), obtaining the calculation formula, y = kx + b. Here, k is the slope of the linear fitting curve, and b is the intercept.
2. Substitute the absorbance of the sample well into the standard curve to calculate the enzyme activity.

Notes

1. The peroxidase sample to be tested, whether it is pure peroxidase or cell or tissue lysate products, can usually be stored for 1 week at 4ºC, and can be stored for a long term at -70ºC, but the activity of peroxidase will significantly decrease after storage at -20ºC.
2. Hydrogen peroxide is unstable, and the precise concentration of hydrogen peroxide should be determined by the method described in this manual.
3. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.